

(FILE 'HOME' ENTERED AT 12:42:38 ON 11 MAR 2005)

FILE 'MEDLINE, BIOSIS, EMBASE' ENTERED AT 12:43:05 ON 11 MAR 2005

L1	708 S G PROTEIN COUPLED RECEPTOR AND SOMATOSTATIN
L2	4 S L1 AND FLIPR
L3	2 DUP REMOVE L2 (2 DUPLICATES REMOVED)
L4	15 S L1 AND SCREEN
L5	7 DUP REMOVE L4 (8 DUPLICATES REMOVED)

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(FILE 'HOME' ENTERED AT 09:33:05 ON 11 MAR 2005)

FILE 'STNGUIDE' ENTERED AT 09:33:18 ON 11 MAR 2005

FILE 'HOME' ENTERED AT 09:33:22 ON 11 MAR 2005

FILE 'MEDLINE, CAPLUS, BIOSIS' ENTERED AT 09:33:33 ON 11 MAR 2005

L1	33478 S G PROTEIN COUPLED RECEPTOR
L2	128623 S FUSION PROTEIN
L3	30732 S CHIMERIC PROTEIN
L4	67069 S CHIMERIC AND (PROTEIN OR RECEPTOR)
L5	3660 S CHIMERIC RECEPTOR
L6	345 S FLIPR
L7	1585 S L1 AND (L2 OR L3 OR L5)
L8	641 S L7 AND (SCREEN OR DETECT OR LIGAND)
L9	70 S L7 AND (SCREEN OR DETECT)
L10	54 DUP REMOVE L9 (16 DUPLICATES REMOVED)

=>

L5 ANSWER 7 OF 7 MEDLINE on STN DUPLICATE 4  
 ACCESSION NUMBER: 97131607 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 8977118  
 TITLE: Characterization of a human gene related to genes encoding **somatostatin** receptors.  
 AUTHOR: Kolakowski L F Jr; Jung B P; Nguyen T; Johnson M P; Lynch K R; Cheng R; Heng H H; George S R; O'Dowd B F  
 CORPORATE SOURCE: Department of Pharmacology, University of Texas Health Science Center at San Antonio, 78284, USA.  
 SOURCE: FEBS letters, (1996 Dec 2) 398 (2-3) 253-8.  
 Journal code: 0155157. ISSN: 0014-5793.  
 PUB. COUNTRY: Netherlands  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 OTHER SOURCE: GENBANK-U71092; GENBANK-U77953  
 ENTRY MONTH: 199701  
 ENTRY DATE: Entered STN: 19970219  
 Last Updated on STN: 20000303  
 Entered Medline: 19970131

AB We report the identification of a gene, named SLC-1(1), encoding a novel **G protein-coupled receptor** (GPCR).  
 A customized search procedure of a database of expressed sequence tags (dbEST) retrieved a human cDNA sequence that partially encoded a GPCR. A genomic DNA fragment identical to the cDNA was obtained and used to **screen** a library to isolate the full-length coding region of the gene. This gene was intronless in its open reading frame, and encoded a receptor of 402 amino acids, and shared ~40% amino acid identity in the transmembrane (TM) regions to the five known human **somatostatin** receptors. Northern blot analysis revealed that SLC-1 is expressed in human brain regions, including the forebrain and hypothalamus. Expression in the rat was highest in brain, followed by heart, kidney, and ovary. Expression of SLC-1 in COS-7 cells failed to show specific binding to radiolabelled Tyr1-**somatostatin**-14, naloxone, bremazocine, 1,3-di(2-tolyl)-guanidine (DTG), or haloperidol. A repeat polymorphism of the form (CA)<sub>n</sub> was discovered in the 5'-untranslated region (UTR) of the gene and SLC-1 was mapped to chromosome 22, q13.3.

L10 ANSWER 51 OF 54 MEDLINE on STN  
ACCESSION NUMBER: 1999288192 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 10334841  
TITLE: Chimeric G proteins allow a high-throughput signaling assay of Gi-coupled receptors.  
AUTHOR: Coward P; Chan S D; Wada H G; Humphries G M; Conklin B R  
CORPORATE SOURCE: Gladstone Institute of Neurological Disease, University of California, San Francisco, California 94141-9100, USA.  
SOURCE: Analytical biochemistry, (1999 Jun 1) 270 (2) 242-8.  
Journal code: 0370535. ISSN: 0003-2697.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199907  
ENTRY DATE: Entered STN: 19990715  
Last Updated on STN: 20000303  
Entered Medline: 19990706

AB **G-protein-coupled receptors** are a major target for potential therapeutics; yet, a large number of these receptors couple to the Gi pathway, generating signals that are difficult to **detect**. We have combined chimeric G proteins, automated sample handling, and simultaneous 96-well fluorometric imaging to develop a high-throughput assay system for Gi signaling. The chimeric G proteins alter receptor coupling so that signaling can occur through Gq and result in mobilization of intracellular calcium stores. An automated signaling assay device, the fluorometric imaging plate reader (FLIPR), can simultaneously measure this response in real time in 96-well microplates, allowing two people to process more than 10,000 points per day. We used the chimeric G protein/FLIPR system to characterize signaling by the Gi-coupled human opioid receptors. We show that the mu, delta, and kappa opioid receptors and the related nociceptin receptor, ORL1, each couple to Galphaqi5, Galphaqo5, and Galpha16 (Galphaqi5 and Galphaqo5 refer to Galphaq proteins containing the five carboxyl-terminal amino acids from Galphai and Galphao, respectively) and that different receptor/G protein combinations show different levels of maximal activation. We tested 31 opioid ligands for agonist activity at the opioid receptors (124 ligand-receptor combinations); all 31 activated at least one receptor type, and several activated multiple receptors with differing potencies. This high-throughput assay could be useful for dissecting the complex ligand-receptor relationships that are common in nature.  
Copyright 1999 Academic Press.

ACCESSION NUMBER: 1999:244759 CAPLUS  
 DOCUMENT NUMBER: 130:276725  
 TITLE: A yeast cell system for screening for effectors of  
**G protein-coupled**  
**receptors** and their interaction with G  
 proteins  
 INVENTOR(S): Broach, James R.; Manfredi, John P.; Paul, Jeremy I.;  
 Truehart, Joshua; Klein, Christine A.; Murphy, Andrew  
 J. M.; Xu, Jun; Benegal, Anupama N.  
 PATENT ASSIGNEE(S): Cadus Pharmaceutical Corporation, USA  
 SOURCE: PCT Int. Appl., 162 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 9  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9918211	A1	19990415	WO 1998-US21168	19981007
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE,				
DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE,				
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW,				
MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR,				
TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES,				
FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI,				
CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6864060	B1	20050308	US 1997-946298	19971007
CA 2305204	AA	19990415	CA 1998-2305204	19981007
AU 9897911	A1	19990427	AU 1998-97911	19981007
EP 1021538	A1	20000726	EP 1998-952143	19981007
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,				
IE, SI, LT, LV, FI, RO				
JP 2001519157	T2	20011023	JP 2000-515008	19981007
US 2003166143	A1	20030904	US 2002-277607	20021022

## PRIORITY APPLN. INFO.:

US 1997-946298	A1	19971007
US 1993-41431	B2	19930331
US 1994-190328	B2	19940131
US 1994-309313	B2	19940920
US 1994-322137	A2	19941013
US 1995-463181	B2	19950605
US 1996-582333	A2	19960117
US 1996-689172	B2	19960806
WO 1998-US21168	W	19981007
US 1998-109902P	P	19981125
US 1998-201396	B1	19981130

AB Transgenic *Saccharomyces cerevisiae* that have mammalian **G protein-coupled receptors** functionally integrated into yeast signaling pathways and that can be used to **screen** for effectors of the receptor are described. The receptor is integrated into the cell membrane using the  $\alpha$  factor leader sequence. Integration of signalling is achieved using host cells expressing a gene for an analog, such as a **fusion protein** or amino acid-substituted variant, of the cognate mammalian G protein subunit. This also allows for the screening of modulators of the interaction of the receptor and the G protein. Specifically, the invention provides novel yeast cells which express a heterologous **G protein coupled receptor** and mutant and/or chimeric G protein subunit mols. which serve to functionally integrate the heterologous into the pheromone signaling pathway of the yeast cell. Functioning of the pathway is demonstrated by induction of a reporter gene. Critical regions of mammalian  $G\alpha$  subunits involved in  $\beta\gamma$  dimer binding were identified by mutational anal. and this information was used to design **fusion proteins** that effectively sequestered the yeast STE4/STE18

$\beta\gamma$  dimer. A **fusion protein** of mammalian  
Gal2 and yeast GPA1 showed better binding of the STE4/STE18  
 $\beta\gamma$  dimer than did Gal2. A number of expression strains  
carrying different combinations of G protein variants were constructed.  
Genes for receptors are transformed into this panel of strains and  
reporter gene expression is used to identify the best host strain.  
Successful expression of a number of receptor genes in yeast is demonstrated.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 45 OF 54 MEDLINE on STN  
ACCESSION NUMBER: 2001069343 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 10924501  
TITLE: The metabotropic GABAB réceptor directly interacts with the activating transcription factor 4.  
AUTHOR: Nehring R B; Horikawa H P; El Far O; Kneussel M; Brandstatter J H; Stamm S; Wischmeyer E; Betz H; Karschin A  
CORPORATE SOURCE: Department of Molecular Neurobiology of Signal Transduction, Max Planck Institute for Biophysical Chemistry, 37070 Gottingen, Germany.  
SOURCE: Journal of biological chemistry, (2000 Nov 10) 275 (45) 35185-91.  
Journal code: 2985121R. ISSN: 0021-9258.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200101  
ENTRY DATE: Entered STN: 20010322  
Last Updated on STN: 20010322  
Entered Medline: 20010104

AB **G protein-coupled receptors**

regulate gene expression by cellular signaling cascades that target transcription factors and their recognition by specific DNA sequences. In the central nervous system, heteromeric metabotropic gamma-aminobutyric acid type B (GABA(B)) receptors through adenylyl cyclase regulate cAMP levels, which may control transcription factor binding to the cAMP response element. Using yeast-two hybrid **screens** of rat brain libraries, we now demonstrate that GABA(B) receptors are engaged in a direct and specific interaction with the activating transcription factor 4 (ATF-4), a member of the cAMP response element-binding protein /ATF family. As confirmed by pull-down assays, ATF-4 associates via its conserved basic leucine zipper domain with the C termini of both GABA(B) receptor (GABA(B)R) 1 and GABA(B)R2 at a site which serves to assemble these receptor subunits in heterodimeric complexes. Confocal fluorescence microscopy shows that GABA(B)R and ATF-4 are strongly coclustered in the soma and at the dendritic membrane surface of both cultured hippocampal neurons as well as retinal amacrine cells in vivo. In oocyte coexpression assays short term signaling of GABA(B)Rs via G proteins was only marginally affected by the presence of the transcription factor, but ATF-4 was moderately stimulated in response to receptor activation in in vivo reporter assays. Thus, inhibitory metabotropic GABA(B)Rs may regulate activity-dependent gene expression via a direct interaction with ATF-4.

L10 ANSWER 46 OF 54 MEDLINE on STN

ACCESSION NUMBER: 2001193745 MEDLINE

DOCUMENT NUMBER: PubMed ID: 11216654

TITLE: Combined modification of intracellular and extracellular loci on human gonadotropin-releasing hormone receptor provides a mechanism for enhanced expression.

AUTHOR: Maya-Nunez G; Janovick J A; Conn P M

CORPORATE SOURCE: Oregon Regional Primate Research Center and Department of Physiology and Pharmacology, Oregon Health Sciences University, Beaverton, USA.

CONTRACT NUMBER: HD-18185 (NICHD)

HD-19899 (NICHD)

RR-00163 (NCRR)

+

SOURCE: Endocrine, (2000 Dec) 13 (3) 401-7.

Journal code: 9434444. ISSN: 0969-711X.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200104

ENTRY DATE: Entered STN: 20010410

Last Updated on STN: 20010410

Entered Medline: 20010405

AB The mammalian gonadotropin-releasing hormone (GnRH) receptor (GnRH-R) has been a therapeutic target for human and animal medicine. This receptor is a unique **G-protein-coupled receptor** that lacks the intracellular C-terminal domain commonly associated with this family. Development of highthrough put **screens** for agents active in humans has been hampered by low expression levels of the hGnRH-R in cellular models. Two sites have attracted the interest of laboratories studying regulation of expression. The chimeric addition of the C-terminal tail from catfish GnRH-R (cfGnRH-R) to the rat GnRH-R significantly augmented receptor expression in GH3 cells. In addition, rodent GnRH-R contains 327 amino acids, but cow, sheep, and human GnRH-R (hGnRH-R) contain 328 residues, the "additional" residue being a Lys 191. Deletion of Lys 191 (del 191) from the hGnRH-R resulted in increased receptor expression levels and decreased internalization rates in both COS-7 and HEK 293 cells. In this study, the combined effect of the addition of the C-tail from cfGnRH-R and deletion of the Lys 191 from the hGnRH-R was compared to expression of the wild-type (WT) or either alteration alone in a transient expression system using primate cells. The altered receptor (hGnRH-R[del 191]-C-tail) showed significantly increased receptor expression at the cell surface compared with the WT or either modification alone. The inositol phosphate response to stimulation was also significantly elevated in response to GnRH agonist. After treatment with a GnRH agonist, the altered receptors showed a slower internalization rate. The homologous steady-state regulation of the WT and the altered receptors was similar, although the response of the altered receptors was significantly decreased. These results suggest that the conformational change in the receptor as a result of the deletion of Lys 191 and the addition of the C-terminus tail substantially increased the steady-state receptor expression and decreased internalization and homologous regulation. Because the effects on expression are greater than additive, it appears that these alterations exert their effects by differing means. These techniques for expression of the hGnRH-R in transfected mammalian cells provide the basis for a therapeutic **screen** for GnRH analogs, agonists, and antagonists of the hGnRH.

L10 ANSWER 47 OF 54 MEDLINE on STN

ACCESSION NUMBER: 2001018413 MEDLINE

DOCUMENT NUMBER: PubMed ID: 10907092

TITLE: Cell-based, high-content **screen** for receptor internalization, recycling and intracellular trafficking.

AUTHOR: Ghosh R N; Chen Y T; DeBiasio R; DeBiasio R L; Conway B R; Minor L K; Demarest K T

CORPORATE SOURCE: Cellomics Inc., Pittsburgh, PA, USA.

SOURCE: BioTechniques, (2000 Jul) 29 (1) 170-5.

Journal code: 8306785. ISSN: 0736-6205.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200011

ENTRY DATE: Entered STN: 20010322

Last Updated on STN: 20010322

Entered Medline: 20001109

AB A variety of physiologically important receptors are internalized and then recycled back to the plasma membrane by the endocytic recycling compartment. These include the transferrin receptor and many **G-protein coupled receptors** (GPCRs). The internalization of GPCRs is a result of agonist stimulation. A cell-based fluorescent imaging assay is described that **detects** and quantifies the presence of fluorescently labeled receptors and macromolecules in the recycling compartment. This High Content Screening application is conducted on the ArrayScan II System that includes fluorescent reagents, imaging instrumentation and the informatics tools necessary to **screen** for compounds that affect receptor internalization, recycling and GPCR activation. We demonstrate the Receptor Internalization and Trafficking application by quantifying (i) the internalization and recycling of the transferrin receptor using a fluorescently labeled ligand and (ii) the internalization of a physiologically functional model GPCR, a GFP-parathyroid hormone receptor chimera. These assays give high signal-to-noise ratios, broad dynamic ranges between stimulated and unstimulated conditions and low variability across different screening runs. Thus, the Receptor Internalization and Trafficking application, in conjunction with the ArrayScan II System, forms the basis of a robust, information-rich and automated **screen** for GPCR activation.

ACCESSION NUMBER: 1999:244759 CAPLUS  
 DOCUMENT NUMBER: 130:276725  
 TITLE: A yeast cell system for screening for effectors of  
**G protein-coupled**  
**receptors** and their interaction with G  
 proteins  
 INVENTOR(S): Broach, James R.; Manfredi, John P.; Paul, Jeremy I.;  
 Truehart, Joshua; Klein, Christine A.; Murphy, Andrew  
 J. M.; Xu, Jun; Benegal, Anupama N.  
 PATENT ASSIGNEE(S): Cadus Pharmaceutical Corporation, USA  
 SOURCE: PCT Int. Appl., 162 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 9  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9918211	A1	19990415	WO 1998-US21168	19981007
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6864060	B1	20050308	US 1997-946298	19971007
CA 2305204	AA	19990415	CA 1998-2305204	19981007
AU 9897911	A1	19990427	AU 1998-97911	19981007
EP 1021538	A1	20000726	EP 1998-952143	19981007
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2001519157	T2	20011023	JP 2000-515008	19981007
US 2003166143	A1	20030904	US 2002-277607	20021022
PRIORITY APPLN. INFO.:			US 1997-946298	A1 19971007
			US 1993-41431	B2 19930331
			US 1994-190328	B2 19940131
			US 1994-309313	B2 19940920
			US 1994-322137	A2 19941013
			US 1995-463181	B2 19950605
			US 1996-582333	A2 19960117
			US 1996-689172	B2 19960806
			WO 1998-US21168	W 19981007
			US 1998-109902P	P 19981125
			US 1998-201396	B1 19981130

AB Transgenic *Saccharomyces cerevisiae* that have mammalian **G protein-coupled receptors** functionally integrated into yeast signaling pathways and that can be used to **screen** for effectors of the receptor are described. The receptor is integrated into the cell membrane using the  $\alpha$  factor leader sequence. Integration of signalling is achieved using host cells expressing a gene for an analog, such as a **fusion protein** or amino acid-substituted variant, of the cognate mammalian G protein subunit. This also allows for the screening of modulators of the interaction of the receptor and the G protein. Specifically, the invention provides novel yeast cells which express a heterologous **G protein coupled receptor** and mutant and/or chimeric G protein subunit mols. which serve to functionally integrate the heterologous into the pheromone signaling pathway of the yeast cell. Functioning of the pathway is demonstrated by induction of a reporter gene. Critical regions of mammalian  $G\alpha$  subunits involved in  $\beta\gamma$  dimer binding were identified by mutational anal. and this information was used to design **fusion proteins** that effectively sequestered the yeast STE4/STE18

L10 ANSWER 44 OF 54 MEDLINE on STN  
ACCESSION NUMBER: 2001074367 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 10958799  
TITLE: The **G protein-coupled receptor** CL1 interacts directly with proteins of the Shank family.  
AUTHOR: Tobaben S; Sudhof T C; Stahl B  
CORPORATE SOURCE: Max Planck-Institute for Experimental Medicine, 37075 Gottingen, Germany.  
SOURCE: Journal of biological chemistry, (2000 Nov 17) 275 (46) 36204-10.  
Journal code: 2985121R. ISSN: 0021-9258.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-AF159046; GENBANK-AF159047; GENBANK-AF159048  
ENTRY MONTH: 200012  
ENTRY DATE: Entered STN: 20010322  
Last Updated on STN: 20010322  
Entered Medline: 20001229

AB PDZ domains play a pivotal role in the synaptic localization of ion channels, receptors, signaling enzymes, and cell adhesion molecules. These domains mediate protein-protein interactions via the recognition of a conserved sequence motif at the extreme C terminus of their target proteins. By means of a yeast two-hybrid **screen** using the C terminus of the G protein-coupled alpha-latrotoxin receptor CL1 as bait, three PDZ domain proteins of the Shank family were identified. These proteins belong to a single protein family characterized by a common domain organization. The PDZ domain is highly conserved among the family members, significantly different from other known PDZ domains, and specifically binds to the C terminus of CL1. Shank1 and CL1 are expressed primarily in brain, and both proteins co-enrich in the postsynaptic density. Furthermore, Shank1 induces a clustering of CL1 in transfected cells, strongly supporting an interaction of both proteins in vivo.

$\beta$ y dimer. A **fusion protein** of mammalian  
Gal2 and yeast GPA1 showed better binding of the STE4/STE18  
 $\beta$ y dimer than did Gal2. A number of expression strains  
carrying different combinations of G protein variants were constructed.  
Genes for receptors are transformed into this panel of strains and  
reporter gene expression is used to identify the best host strain.  
Successful expression of a number of receptor genes in yeast is demonstrated.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT